# Volatile Cues Influence the Response of *Rhopalosiphum padi* (Homoptera: Aphididae) to Barley Yellow Dwarf Virus–Infected Transgenic and Untransformed Wheat

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**ABSTRACT** The attractiveness of *Barley yellow dwarf luteovirus* (BYDV)–infected wheat plants to *Rhopalosiphum padi* L. was evaluated under laboratory conditions. Two untransformed wheat varieties, virus-susceptible Lambert and virus-tolerant Caldwell, and one transgenic wheat genotype (103.1J) derived from Lambert and expressing the BYDV *coat protein* gene, were tested in three bioassays. First, *R. padi* responses to BYDV-infected or noninfected Lambert and Caldwell were evaluated. Significantly more aphids settled onto virus-infected than noninfected plants when aphids were able to contact the leaves. Second, aphid responses to headspace from virus-infected or noninfected Lambert and Caldwell were tested. Significantly more aphids congregated on screens above headspace of BYDV-infected plants than above headspace of noninfected plants of both varieties. Third, aphid responses to headspace from virus-infected or noninfected and sham-inoculated (exposed to nonviruliferous aphids) Lambert and 103.1J plants were examined. Significantly more aphids congregated on screens above BYDV-infected than above noninfected or sham-inoculated Lambert. No significant differences in *R. padi* preferences for headspace above BYDV-infected compared with noninfected or sham-inoculated 103.1J plants were observed. The concentration of volatiles extractable from whole plant headspace was greater on BYDV-infected Lambert than on BYDV-infected 103.1J, noninfected, or sham-inoculated plants of either genotype. This is the first report of volatile cues associated with BYDV infection in wheat plants influencing the behavior of the vector *R. padi*. Additionally, these findings show for the first time that transgenic virus resistance in wheat can indirectly influence the production of volatiles making virus-infected plants less attractive or arrestant to aphids than are infected untransformed plants.

**KEY WORDS** virus-induced volatiles, virus vectors, insect behavior, host plant resistance, coat protein–mediated resistance

THE BIRD CHERRY-OAT APHID, *Rhopalosiphum padi* L., is one of the most serious insect pests of cereals worldwide and one of the main vectors of *Barley yellow dwarf virus* (BYDV) (Gildow and Rochow 1983). In Idaho, *R. padi* is one of the most numerous and economically important aphids on winter and spring wheat, *Triticum aestivum* L. (Forster and Rochow 1983, Bishop and Sandvol 1984, Schotzko and Bosque-Pérez 2000), damaging hosts both by direct feeding and by transmitting BYDV (Stern 1967). BYDV is a member of the family Luteoviridae, genus *Luteovirus* (van Regenmortel et al. 2000), and is transmitted in a persistent-circulative manner by *R. padi* and 25 other aphid species in North America (Halbert and Voegtlin 1995). BYDV disease involves a complex interaction between plant, virus, and aphid vectors (Irwin and Tresh 1990).

Virus-infected plants have been shown to affect the biology of aphid vectors (Macias and Mink 1969, Ajayi and Dewar 1983, Blua and Perring 1992, Eckel and Lampert 1996). Most of the available literature suggests that plants infected with virus are more favorable to insect vectors than their healthy counterparts, decreasing their developmental periods and increasing their growth rates, longevity, and/or fecundity (Kennedy 1951, Laurema et al. 1966, Araya and Foster 1987, Fereres et al. 1989, Quiroz et al. 1991, Jiménez-Martínez et al. 2004). Virus-infected plants also may have deleterious effects on aphid life history (MacKinson 1960, Lowe and Strong 1963, Markkula and Laurema 1964, Michels et al. 1994).

Volatile compounds emitted by plants are involved in plant herbivore interactions (Pickett et al. 1992).

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Cereal crops such as wheat and oat are known to produce volatiles (Buttery et al. 1982, 1985, Hamilton-Kemp and Andersen 1984, 1986). Plant volatiles stimulate behavioral responses of aphids such as *R. padi* (Pettersson 1993, 1994, Pettersson et al. 1995, Quiroz et al. 1997, Quiroz and Niemeyer 1998). Other aphid species are reported to respond preferentially to plants infected with virus. Baker (1960) and Macias and Mink (1969) reported a preference of apterous *Myzus persicae* (Sulzer) for beet leaves infected with *Beet western yellows virus* or *Beet yellows virus* compared with symptomless plants or those infected with either *Beet mosaic virus* or *Beet curly top virus*. Blua and Perring (1992) found *Aphis gossypii* (Glover) preferentially responded to plants infected with *Zucchini yellow mosaic virus* after 2 wk of infection compared with plants after 4 wk of infection. Eckel and Lampert (1996) reported that, under field conditions, aphids preferentially responded to tobacco plants infected with *Tobacco etch virus* rather than flue-cured tobacco plants. Castle et al. (1998) detected a preferential colonization by *M. persicae* of *Potato leafroll luteovirus* (PLRV)–infected potato plants over healthy plants or *Potato virus X* (PVX)– or *Potato virus Y* (PVY)–infected potato plants. More recently, Eigenbrode et al. (2002) reported that potato plants infected with PLRV release volatiles, and as a result, they are preferred by *M. persicae* compared with healthy or PVX- or PVY-infected plants under laboratory conditions.

We hypothesized that wheat plants infected with *Barley yellow dwarf luteovirus* release volatiles that influence the response of *R. padi.* The overall objective of this study was to determine if volatile cues affect the response of *R. padi* to BYDV-infected transgenic and untransformed wheat plants. Our specific objectives were to determine the response of *R. padi* to BYDV-infected untransformed wheat versus noninfected control plants, determine the response of *R. padi* to BYDV-infected transgenic and untransformed wheat plants compared with noninfected or sham-inoculated plants, determine if volatile cues mediate aphid responses, and identify possible volatile components influencing *R. padi* responses.

## Materials and Methods

**Plant Material, Virus Isolate, and Aphids.** Untransformed plants of the BYDV-tolerant variety 'Caldwell' (Patterson et al. 1982) and the BYDV-susceptible variety 'Lambert' (Zemetra et al. 1995) were tested. We also tested third-generation transgenic soft white winter wheat plants from genotype 103.1J (derived from the parental variety Lambert) that express the BYDV (PAV serotype) *coat protein* (*CP*) gene (Hansen et al. 1998). This transgenic line was selected because, in previous experiments, it showed low virus titer compared with Lambert and Caldwell (Jiménez-Martínez et al. 2004). Seeds from each genotype were planted in 10.2-cm plastic pots filled with soil mixture (6:1 ratio Sunshine mix #1; Sun-Gro Horticulture, Bellevue, WA) and sand and kept in a growth chamber until they reached the two- to three-leaf stage (Zadoks 12–13) (Zadoks et al. 1974); then they could be inoculated. Beginning at the two- to three-leaf stage, plants were fertilized with a soluble N-P-K fertilizer (20:20:20) every other week. A Washington State isolate of BYDV-PAV maintained by mass transfer of *R. padi* in barley plants (variety 'Sprinter') was used for virus inoculation of plants.

Aphids were obtained from a virus-free colony of *R. padi* (Idaho clone) maintained on Sprinter barley in environmental growth chambers (Mod-1–36VLX; Percival Scientific, Perry, IA) at 20 ± 1°C and a photoperiod of 16:8 (L:D) at the University of Idaho, Moscow, ID. Before experimental use, aphids were preconditioned for one generation in small subcolonies on Sprinter barley (Schotzko and Smith 1991).

**Confirmation of Transgenic Status of 103.1J Plants.** A polymerase chain reaction (PCR) protocol, described by Jiménez-Martínez et al. (2004), was used to screen 103.1J plants to ensure that each test plant carried the BYDV *CP* gene. PCR was performed using 20-mer primers (Invitrogen, Rockville, MD) designed using the "Primer Designer for Windows" (version 2.0, Scientific and Educational Software). Primers were designed to amplify a 499-base fragment of the BYDV *CP* gene. Only PCR-positive plants were used in the tests.

**Inoculation of Plants with BYDV.** Plant virus inoculation took place at the two- to three-leaf stage. Plants were inoculated with BYDV using viruliferous fourth-instar nymphs and adult apterae of *R. padi.* Ten aphids per plant were confined using a fine cage made with dialyzing tubing (≈2.5 cm in length by 1 cm in diameter; Spectrum Laboratories, Rancho Dominguez, CA). The cage was secured to the plant with a foam stopper at both ends to provide ventilation and prevent aphids from escaping. An inoculation access period of 72 h was used to ensure virus inoculation of all plants; afterward, aphids were removed and killed. Plants were kept in environmental growth chambers at 20 ± 1°C for 15 d after inoculation.

**Sham-Inoculated Plants.** Insect feeding may induce resistance in plants and potentially affect the response of insects subsequently exposed to such plants (Karban and Baldwin 1997). To minimize these confounding effects in the interpretation of results, we also used sham-inoculated plants. To obtain sham-inoculated plants, the procedure used to produce BYDV-infected plants described above was used, but aphids were virus-free instead of viruliferous. Aphids were obtained from a nonviruliferous colony kept in a separate laboratory at the conditions described earlier for the viruliferous colony. Sham-inoculated plants were only used for the last bioassay, where we tested transgenic and untransformed plants.

**Bioassays.** Three sets of bioassays were conducted to determine if volatile cues influence the responses of *R. padi* to BYDV-infected plants. Bioassays were established in a laboratory maintained at 20 ± 1°C. In all cases, aphids were starved for 1 h before bioassays, and 40 adult apterous nonviruliferous *R. padi* were placed in the center of an arena. The arena was darkened to eliminate visual cues that could affect aphid behavior.

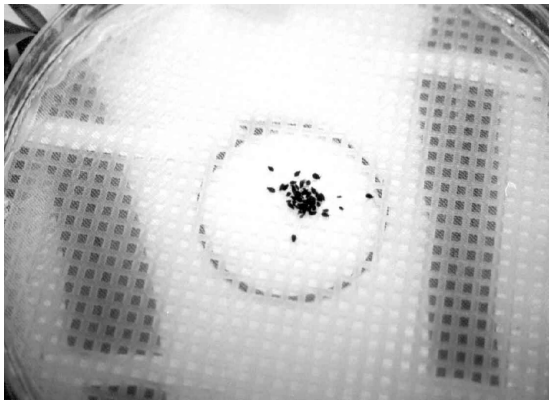

**Fig. 1.** Arena used to conduct the headspace volatiles test. Aphids on screen above leaves were unable to contact leaf surfaces, and leaves remained attached to plants during bioassay.

Aphid locations were recorded at pre-established time intervals. During an observation, the arena was opened and illuminated with a red light for a few seconds.

**Aphid Response to BYDV-Infected Versus Noninfected Untransformed Varieties 'Lambert' and 'Caldwell'.** The response of aphids to BYDV-infected versus noninfected plants of Lambert and Caldwell was tested in separate arenas. There were 10 replicates for each comparison per variety. The arena was made from a 150-mm-diameter polystyrene petri dish. The bottom of the petri dish was lined with 150-mm-diameter filter paper (Whatman), which was in contact with wheat leaves. The leaves, attached to the test plants, were positioned horizontally in the dish, equally distant from the center opposite to one another. Three leaves from each of two treatments (infected or noninfected) were used to produce a dual-choice test. Aphids could touch the leaves with their stylets or tarsi. Aphid locations on or near the leaves (≈1 cm on either side of leaves) were recorded every 10 min for a 2-h period. The total number of aphids tested for the entire bioassay was 800. Each test was analyzed as a separate experiment comparing the number of aphids on each treatment and using a generalized linear model, assuming a binomial distribution with a logit link function (PROC GENMOD; SAS Institute 1990). Aphids not located on either treatment were excluded from the analysis.

**Aphid Response to Headspace above BYDV-Infected Versus Noninfected Untransformed Varieties 'Lambert' and 'Caldwell.'** The responses of aphids to headspace volatiles from BYDV-infected or noninfected Lambert plants and to BYDV-infected or noninfected Caldwell plants were tested in separate arenas. There were 10 replicates for each comparison per variety. An arena (Fig. 1) was made from a 150-mm-diameter polystyrene petri dish and fitted with a false floor of 1-mm polyethylene mesh screen (Eigenbrode et al. 2002). Three leaves, still attached to plants, were positioned ≈4 mm beneath the screen floor opposite one another. Aphids on the screen could walk freely but could not touch the leaves with their stylets or tarsi. The distance from leaf to screen was selected to expose aphids to headspace volatiles (volatiles near the plants) when no visual, gustatory, or contact cues were present. This short distance was also used to minimize the potential confounding effect of volatiles cues from the two sets of leaves mixing within the arena. To reduce this further, the volume above the test screen was sufficiently large (750 $cm^2$) to prevent saturation with plant volatiles from two treatments during the test (Eigenbrode et al. 2002). Aphid locations were recorded at 10 and 20 min and every 20 min thereafter for a 2-h period. An aphid was recorded as responding to the leaves if it was directly above any leaf part. The total number of aphids tested for the entire bioassay was 800. The mean number of aphids on each of the two treatments (infected or noninfected) was compared using a generalized linear model, assuming a binomial distribution with a logit link function (PROC GENMOD; SAS Institute 1990).

**Aphid Response to Headspace above BYDV-Infected Versus Noninfected and Versus Sham-Inoculated Untransformed and Transgenic Plants.** Wheat plants from the untransformed variety Lambert and the transgenic genotype 103.1J were tested in the bioassay. The arena and method used to record aphid responses were as described in the previous bioassay. The comparisons tested were as follows: BYDV-infected Lambert versus noninfected Lambert, BYDV-infected Lambert versus sham-inoculated Lambert, noninfected Lambert versus sham-inoculated Lambert, BYDV-infected 103.1J versus noninfected 103.1J, BYDV-infected 103.1J versus sham-inoculated 103.1J, and noninfected 103.1J versus sham-inoculated 103.1J. In addition, in a separate test, we compared noninfected 103.1J versus noninfected Lambert. For each comparison, there were six replications. The total number of aphids tested for the entire bioassay was 1,680. Aphid locations at 10 and 20 min and every 20 min thereafter during a 3-h period were compared using a generalized linear model, assuming a binomial distribution with a logit link function (PROC GENMOD; SAS Institute 1990).

**Analysis of Headspace Volatiles.** Headspace volatiles from BYDV-infected, sham-inoculated, and noninfected controls from untransformed and transgenic wheat plants were trapped and collected as described by Eigenbrode et al. (2002). Four intact plants, with roots and the base of the plants wrapped securely with aluminum foil, were enclosed in a glass collection chamber (Analytical Research Systems, Gainesville, FL). Humidified air, prefiltered through activated carbon and Super-Q adsorbent resin (Alltech Associates, Deerfield, IL), was drawn through the chamber for 24 h, at 300 ml/min, exiting through a trap containing 100 mg of Super-Q. Immediately after volatile collection, the entire aerial portion of the plants was removed for determination of fresh and dry weights. Elutant from Super-Q trap was standardized to 400 $\mu$l, and a 1-$\mu$l sample was injected onto a Hewlett-Packard 6890 gas chromatograph with a Hewlett-Packard

**Table 1. Response of apterous *R. padi* during 2 h in test arenas (aphids contacting the leaves) containing BYDV-infected and noninfected wheat leaves from untransformed varieties 'Lambert' and 'Caldwell'**

| Variety | Treatment | Aphid response to treatments per 10 min | | |
|---|---|---|---|---|
| | | Aphids on leaves | Aphids near leaves[a] | Total aphids |
| Lambert | BYDV-infected | 15.2 ± 1.45a | 1.82 ± 0.18 | 17.0 ± 1.39a |
| Lambert | Noninfected | 11.0 ± 1.17b | 1.21 ± 0.11 | 12.2 ± 1.15b |
| *P* | | 0.01 | NS | 0.0054 |
| Caldwell | BYDV-infected | 15.0 ± 1.54a | 1.98 ± 0.23 | 16.9 ± 1.43a |
| Caldwell | Noninfected | 10.8 ± 1.10b | 1.82 ± 0.32 | 12.6 ± 1.27b |
| *P* | | 0.0085 | NS | 0.0110 |

Values are means ± SE.

Means of 40 aphids per test, with observations at 10-min intervals during 2 h and 10 replicates. Means in the same column per each dual choice comparison followed by different letters are significantly different at $P \leq 0.05$. Significance of values is based on a generalized linear model, assuming a binomial distribution with a logit link function.

[a]Aphids near leaves ≈1 cm on either side of leaves.

5973 Mass Selective Detector (Agilent Technologies, Palo Alto, CA). An external standard of terpenol was used to standardize volatile samples. The column was a 30 m by 0.2 mm i.d. HP-1, held at 40°C for 2 min and then heated to 250°C at 10°C/min and held for 10 min. Peaks were identified based on the National Institute of Standards and Technology library mass spectra, and interpretation was based on fragmentation and spectra of available authentic standards. Fresh weight/dry weight ratios were similar for all treatments, and quantities were calculated as nanogram per 100 g of aboveground fresh plant. Separate volatile collections were made from three replicates (each of four wheat plants) for each treatment, and this provided a basis for statistical comparisons. Injections for each replicate were made in triplicate. Total volatile concentration in the headspace from the six treatments was compared using ANOVA. Means were separated by a least significant difference (LSD) procedure ($\alpha$ = 0.05, PROC GLM; SAS Institute 1990). Differences in the concentration of individual components were not compared statistically, but SE were calculated.

**Virus Titer Determination.** Plants were observed for virus symptoms and tested for virus presence or absence with enzyme-linked immunosorbent assay (ELISA) 25 d after inoculation. Double antibody sandwich (DAS) ELISA was used to measure virus titer in BYDV-infected plants as described by Gray et al. (1991) and Jiménez-Martínez et al. (2004). Plates were read at $A_{405\ nm}$ when the absorbance (OD) of the positive controls was approximately two to three times higher than the negative control. Known concentrations of purified virus, diluted in buffer, were included on each plate as controls. Each plate used included duplicate wells containing a dilution series of 50, 100, 500, and 1,000 ng of purified virus, in addition to healthy plant sap (negative check) and BYDV-infected plant sap (positive check). The set of four purified virus standards included on each plate allowed for direct comparisons of absorbance values among plates; therefore, a standard curve that converts absorbance values to virus titer concentrations could be generated. Virus titer data are presented as mean ± SE of nanograms of virus per milliliter of plant sap. Analysis of variance (ANOVA) using a generalized linear model (PROC GLM) followed by a least square means procedure (LSMEANS) was carried out to compare absorbance (OD) and virus titer among genotypes. All analyses were conducted using SAS (SAS Institute 1990).

## Results

**Aphid Responses to BYDV-Infected Versus Noninfected Untransformed Wheat.** We compared the number of apterous *R. padi* on and near wheat leaves of untransformed varieties Lambert and Caldwell infected with BYDV versus noninfected. Significantly more aphids were located on leaves of Lambert infected with BYDV ($P = 0.01$) and Caldwell infected with BYDV ($P = 0.0085$) than were located on noninfected leaves of either variety (Table 1). No significant differences were found between treatments for either variety in the number of aphids located near the leaves (within 1 cm). The total number of aphids (aphids on leaves plus aphids near leaves) responding to plants infected with BYDV versus noninfected ones was also analyzed. Significantly more aphids responded to BYDV-infected wheat leaves than noninfected leaves of Lambert ($P = 0.0054$) and Caldwell ($P = 0.011$; Table 1). Aphid preference for BYDV-infected Lambert leaves was significantly greater at 30 min after the test began and throughout the remainder of the 2-h bioassay (Fig. 2).

**Aphid Responses to Headspace above BYDV-Infected Versus Noninfected Untransformed Wheat.** Significantly more aphids congregated in target areas above leaves of Lambert ($P = 0.0079$) and Caldwell ($P = 0.0259$) infected with BYDV than above leaves of noninfected plants of either variety (Table 2).

**Aphid Responses to Headspace above BYDV-Infected Versus Noninfected and Versus Sham-Inoculated Transgenic and Untransformed Wheat Plants.** The response of *R. padi* to headspace above Lambert infected with BYDV was significantly greater than to headspace above noninfected ($P = 0.024$) and sham-inoculated ($P = 0.002$) plants of this variety (Table 3). Aphid preference for BYDV-infected Lambert leaves was significantly greater throughout the duration of the 3-h bioassay (Fig. 3). No significant preferential

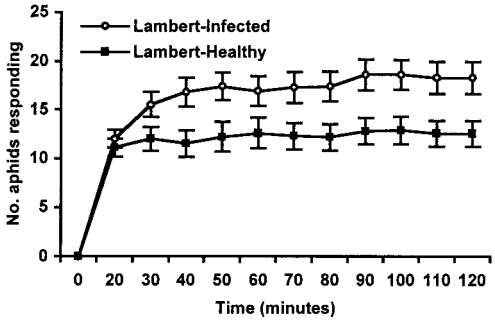


**Fig. 2.** Preferential response of *R. padi* apterae after 2 h in a dual choice test comparing BYDV-infected versus noninfected plants of the wheat variety Lambert, when aphids had contact with the leaves. Error bars are SEM of the total number of aphids on and near leaves responding to either treatment. Comparison was significant based on a generalized linear model, assuming a binomial distribution with a logit link function ($P = 0.0054$).

responses were found to headspace of noninfected versus sham-inoculated Lambert plants (Table 3).

*Rhopalosiphum padi* showed no preference for headspace above wheat plants of the transgenic genotype 103.1J infected with BYDV compared with headspace above noninfected or sham-inoculated plants of this genotype (Table 3). A similar response was observed throughout the duration of the 3-h bioassay (Fig. 4). In a separate test, no significant preference was detected for headspace above noninfected Lambert versus noninfected 103.1J wheat plants (Table 3).

**Analysis of Headspace Volatiles from Untransformed and Transgenic Wheat Plants.** Twenty components were identified or partly identified in headspace extracts of untransformed and transgenic wheat plants (Table 4). Six of the components have previously been reported from wheat plant headspace (Quiroz et al. 1997) or volatile extracts of macerated wheat tissue (Hamilton-Kemp and Andersen 1984, 1996, Buttery et al. 1985) ([Z]-3-hexenyl acetate, naphthalene, nonanal, decanal, pentadecane, and hexadecane). Among those not previously reported from wheat were several *n*-alkanes and methyl-branched alkanes and three sesquiterpenes. In addition, five alkylated aromatic compounds were detected (1-ethyl-2-methylbenzene, 1,2,3-trimethylbenzene, 1,3,5-trimethylbenzene, 4-ethyl-1,2-dimethyl-

**Table 2. Response of apterous *R. padi* to headspace during 2 h in test arenas (aphids not contacting the leaves) containing leaves of BYDV-infected and noninfected plants of untransformed cultivars 'Lambert' and 'Caldwell'**

| Variety | Treatment | Aphids responding to treatments per 20 min |
|---|---|---|
| Lambert | BYDV-infected | 14.1 ± 1.59a |
| Lambert | Non-infected | 10.0 ± 0.65b |
| *P* | | 0.0079 |
| Caldwell | BYDV-infected | 14.0 ± 0.83a |
| Caldwell | Non-infected | 10.5 ± 0.71b |
| *P* | | 0.0259 |

Values are means ± SE.

Means of 40 aphids per test, with observations at 10 and 20 min and every 20 min thereafter during 2 h and 10 replicates. Means in the same column per each dual choice comparison followed by different letters are significantly different at $P \leq 0.05$. Significance of values is based on a generalized linear model, assuming a binomial distribution with a logit link function.

**Table 3. Response of apterous *R. padi* to headspace during 3 h in test arenas (aphids not contacting the leaves) containing leaves of BYDV-infected, noninfected, and sham-inoculated plants of untransformed Lambert and Lambert-derived transgenic wheat genotype 103.1J**

| Genotype | Treatment | Aphids responding to treatments per 20 min |
|---|---|---|
| Lambert | BYDV-infected | 11.7 ± 1.69a |
| Lambert | Noninfected | 6.57 ± 0.59b |
| *P* | | 0.024 |
| Lambert | BYDV-infected | 11.8 ± 1.52a |
| Lambert | Sham-inoculated[a] | 6.30 ± 1.06b |
| *P* | | 0.002 |
| Lambert | Noninfected | 9.27 ± 0.98 |
| Lambert | Sham-inoculated | 8.58 ± 0.82 |
| *P* | | 0.444 |
| Lambert | Noninfected | 5.15 ± 0.45 |
| 103.1J | Noninfected | 5.65 ± 0.73 |
| *P* | | 0.571 |
| 103.1J | BYDV-infected | 8.18 ± 0.66 |
| 103.1J | Noninfected | 6.48 ± 0.77 |
| *P* | | 0.163 |
| 103.1J | BYDV-infected | 7.70 ± 1.10 |
| 103.1J | Sham-inoculated | 6.72 ± 0.74 |
| *P* | | 0.091 |
| 103.1J | Noninfected | 8.80 ± 0.76 |
| 103.1J | Sham-inoculated | 8.35 ± 1.03 |
| *P* | | 0.409 |

Values are means ± SE.

Means of 40 aphids per test, with observations at 10 and 20 min and every 20 min thereafter during 3 h and six replicates. Means on the same column per each dual choice comparison followed by different letters are significantly different at $P \leq 0.05$. Significance of values is based on a generalized linear model, assuming a binomial distribution with a logit link function.

[a]Plants exposed to 10 nonviruliferous aphids 2 wk before bioassays.

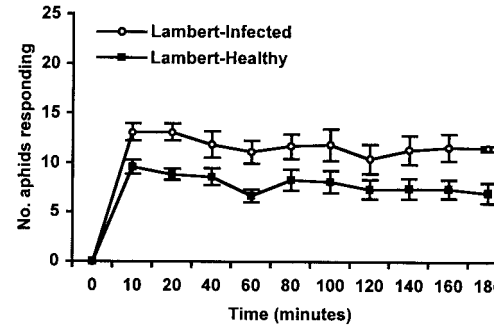


**Fig. 3.** Preferential response of *R. padi* apterae after 3 h in a dual choice test comparing headspace above BYDV-infected versus noninfected plants of the wheat variety Lambert. Aphids were not contacting the leaves. Error bars are SEM of the total number of aphids responding to either treatment. Comparison was significant based on a generalized linear model, assuming a binomial distribution with a logit link function ($P = 0.024$).

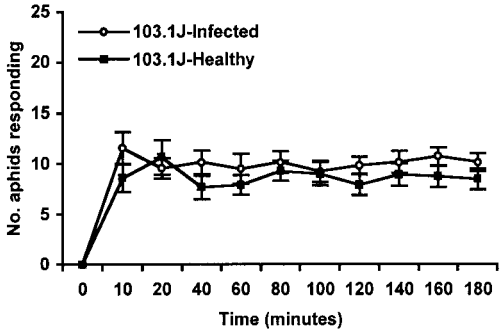


**Fig. 4.** Response of *R. padi* apterae after 3 h in a dual choice test comparing headspace above BYDV-infected versus noninfected plants of the Lambert-derived transgenic genotype 103.1J. Aphids were not contacting the leaves. Error bars are SEM of the total number of aphids responding to either treatment. Comparison was not significant based on a generalized linear model, assuming a binomial distribution with a logit link function ($P = 0.163$).

benzene, and 1,2,4,5-tetramethylbenzene). These and similar compounds typically are anthropogenic and are likely contaminants. Therefore, they have not been included in Table 4. Nonetheless, because biogenic alkylated aromatics have been reported previously from plant volatiles (Kemp et al. 1972, MacLeod et al. 1985, Nair and Burke 1990, Miles et al. 2000, GuangYing et al. 2001), and could have been produced by the plants in our study, we will summarize patterns in their occurrence qualitatively. The concentration of the total extractable gas chromatography-mass spectometry (GC-MS) detectable volatiles in headspace from BYDV-infected Lambert was 2.8- and 3.8-fold greater than in headspace of noninfected and sham-inoculated controls, respectively ($P \leq 0.05$; Table 4). Total concentration of volatiles was approximately similar in headspace of noninfected and sham-inoculated plants (Table 4). Although the same components were present in the headspace of plants from all treatments of Lambert, there were differences in relative composition. Based on nonoverlapping SEs, Lambert infected with BYDV produced three-fold higher concentrations of (Z)-3-hexenyl acetate than noninfected Lambert (Table 4). All other detected compounds (Table 4) had higher mean concentrations in the headspace from infected plants, but SEs overlapped for all but undecane and 2-methyl dodecane. Among the alkylated aromatics, 1-ethyl-2-methylbenzene and 1,2,4,5-tetramethylbenzene concentrations were higher in headspace of noninfected than infected Lambert plants.

The concentrations of GC-MS detectable components in the headspace from the noninfected plants of transgenic genotype 103.1J were similar to those of 103.1J plants infected with BYDV (Table 4). The same 20 components found in Lambert headspace were detected in headspace of 103.1J plants. Based on nonoverlapping SEs, 3-methyl tridecane concentration was higher in the headspace of noninfected 103.1J than in headspace of infected 103.1J. Among the alkylated aromatics detected, 1,2,3-trimethylbenzene concentration was higher in headspace of infected

**Table 4. Headspace volatiles from BYDV-infected, noninfected, and sham-inoculated plants of untransformed Lambert and Lambert-derived transgenic wheat genotype 103.1J**

| Component[b] | Nanograms per 100 g (fresh wt) per 24 h | | | | | |
|---|---|---|---|---|---|---|
| | BYDV-Infected | | Noninfected | | Sham-inoculated[a] | |
| | Lambert | 103.1J | Lambert | 103.1J | Lambert | 103.1J |
| Methoxybenzene | 12 ± 6 | 4 ± 1 | 6 ± 3 | 13 ± 7 | 4 ± 2 | 3 ± 1 |
| (Z)-3-Hexenyl acetate | 3299 ± 446 | 156 ± 156 | 992 ± 992 | 619 ± 501 | 572 ± 96 | 378 ± 230 |
| Undecane | 119 ± 52 | 20 ± 5 | 38 ± 38 | 51 ± 11 | 63 ± 29 | 42 ± 7 |
| Nonanal | 235 ± 177 | 48 ± 27 | 36 ± 36 | 72 ± 29 | 101 ± 19 | 52 ± 13 |
| Napthalene | 72 ± 41 | 8 ± 1 | 17 ± 6 | 16 ± 4 | 26 ± 18 | 18 ± 6 |
| Dodecane | 59 ± 35 | 9 ± 2 | 39 ± 28 | 23 ± 9 | 27 ± 11 | 18 ± 3 |
| Decanal | 218 ± 181 | 49 ± 36 | 32 ± 16 | 62 ± 37 | 83 ± 35 | 31 ± 16 |
| 2-Methyl dodecane[c] | 112 ± 68 | 17 ± 2 | 30 ± 12 | 122 ± 88 | 60 ± 23 | 45 ± 8 |
| Tridecane | 307 ± 175 | 27 ± 1 | 111 ± 41 | 85 ± 8 | 125 ± 93 | 88 ± 32 |
| 2-Methyl tridecane[c] | 593 ± 428 | 68 ± 14 | 248 ± 91 | 185 ± 45 | 236 ± 129 | 158 ± 35 |
| 3-Methyl tridecane[c] | 871 ± 563 | 127 ± 14 | 390 ± 108 | 290 ± 38 | 327 ± 199 | 245 ± 58 |
| Copaene[c] | 183 ± 97 | 3 ± 3 | 77 ± 48 | 29 ± 3 | 10 ± 10 | 41 ± 7 |
| Tetradecane | 443 ± 345 | 21 ± 2 | 141 ± 58 | 56 ± 9 | 89 ± 68 | 64 ± 22 |
| Caryophyllene | 968 ± 407 | 28 ± 7 | 413 ± 229 | 135 ± 12 | 219 ± 126 | 182 ± 27 |
| Unidentified sesquiterpene | 397 ± 176 | 12 ± 9 | 109 ± 55 | 61 ± 25 | 112 ± 99 | 88 ± 32 |
| 9-Methyl nonadecane | 244 ± 142 | 17 ± 4 | 115 ± 56 | 69 ± 6 | 82 ± 60 | 61 ± 17 |
| Pentadecane | 507 ± 331 | 52 ± 7 | 241 ± 104 | 123 ± 25 | 141 ± 60 | 95 ± 13 |
| 2-Methyl pentadecane[c] | 114 ± 85 | 7 ± 1 | 40 ± 22 | 18 ± 3 | 27 ± 16 | 18 ± 5 |
| 3-Methyl pentadecane[c] | 139 ± 95 | 11 ± 3 | 53 ± 27 | 21 ± 1 | 30 ± 16 | 19 ± 5 |
| Hexadecane | 261 ± 135 | 14 ± 6 | 101 ± 62 | 24 ± 1 | 46 ± 19 | 20 ± 5 |
| Total | 9153 ± 2730a | 698 ± 236b | 3229 ± 1680b | 2074 ± 600b | 2380 ± 869b | 1666 ± 228b |

Values are the mean ± SE of three replicate extractions from four plants of each treatment. Values in the last row with the same letter are not significantly different at $\alpha = 0.05$. LSD from an ANOVA $F_{5,12} = 4.91$, $P = 0.01$.

[a] Sham-inoculated plants were exposed to nonviruliferous aphids 2 wk before bioassays.

[b] In order of elution during gas chromatography.

[c] Isomer not determined.

**Table 5. BYDV-PAV absorbance (OD) and virus titer on untransformed Lambert and Caldwell and Lambert-derived transgenic wheat genotype 103.1J**

| Genotype | Treatment | Absorbance ($OD_{405\ nm}$) | Virus titer (ng/ml) |
|---|---|---|---|
| Lambert | Infected | 0.989 ± 0.114a | 543.1 ± 97.9b |
| Lambert | Noninfected | 0.239 ± 0.009b | |
| Lambert | Sham-inoculated[a] | 0.259 ± 0.012b | |
| 103.1J | Infected | 0.366 ± 0.025b | 80.9 ± 21.4a |
| 103.1J | Noninfected | 0.216 ± 0.009b | |
| 103.1J | Sham-inoculated | 0.264 ± 0.017b | |
| Caldwell | Infected | 1.123 ± 0.083a | 657.7 ± 70.9b |
| Caldwell | Noninfected | 0.170 ± 0.015b | |
| Caldwell | Sham-inoculated | ND | |
| *P* | | 0.0001 | 0.0001 |

Values are means ± SE.

Means of 12 plants infected with BYDV. ELISA conducted 25 d after inoculation. Means on the same column followed by the same letter are not significantly different at ($P \leq 0.05$).

[a]Plants exposed to 10 nonviruliferous aphids 2 wk before bioassays.

ND, not done.

103.1J plants than in headspace of noninfected 103.1J plants, and 1,2,4,5-tetramethylbenzene concentration was higher in headspace of noninfected 103.1J.

Sham-inoculated plants of both genotypes had similar concentrations of total headspace volatiles and most of the individual components to noninfected controls (Table 4). Nonanal concentration was higher in sham-inoculated Lambert than in noninfected Lambert but did not differ between sham-inoculated and noninfected 103.1J. Among the alkylated aromatics detected, 1-ethyl-2-methylbenzene concentration was lower in headspace of sham-inoculated than noninfected plants of both genotypes, and 1,2,3-trimethylbenzene was higher in headspace of sham-inoculated 103.1J than in noninfected 103.1J.

The total concentration of volatiles from BYDV-infected Lambert was significantly greater than all other treatments (Table 4), including noninfected and BYDV-infected 103.1J. This pattern also occurred if the alkylated aromatic components were included in the total headspace volatiles.

**Virus Titer on BYDV-Infected Plants.** ELISA tests were done on samples from all genotypes 25 d after inoculation. Lambert and Caldwell had significantly higher absorbance (OD; $P = 0.0001$) and consequently significantly higher virus titer ($P = 0.0001$) compared with transgenic 103.1J (Table 5). Transgenic plants showed moderate resistance to BYDV-PAV, as indicated by the lower virus titer compared with untransformed plants. Working on transgenic genotypes of oat and barley expressing the coat protein of BYDV, McGrath et al. (1997) also reported low ELISA values, indicative of reduced virus titer, on BYDV-infected transgenic plants.

**Relationship Between Virus Titer and Aphid Response.** To explore potential relationships between virus titer and aphid responses, the proportion of aphids responding in a choice test was plotted against virus titer levels for each of 12 plants of Lambert or 103.1J. No significant relationship was detected for infected Lambert, in which plants always had virus titers >245 ng of virus/ml of plant sap (Fig. 5). In contrast, a positive relationship was detected for infected 103.1J. The proportion of aphids responding increased significantly ($P = 0.054$) as virus titer increased from 43 to a maximum of 134 ng of virus/ml of plant sap (Fig. 5).

## Discussion

*Rhopalosiphum padi* preferentially responded to untransformed wheat plants infected with BYDV when in contact with the leaves. More aphids were located on leaves from BYDV-infected plants than on leaves from noninfected control plants. In bioassays in which aphids could not contact the leaves, but could move on a screen directly above the plants, aphids responded similarly. It is not clear if this is a dynamic equilibrium or, if once aphids select a site, their location in the arena does not change for the remaining of the bioassay. Further studies are required to clarify this. We conclude that volatile cues were involved in the aphid preferential response for virus-infected compared with noninfected or sham-inoculated Lambert plants. We conclude this because the preferential responses took place in the dark, precluding visual orientation, and occurred when aphids were prevented from contacting the leaves, preventing gusta-

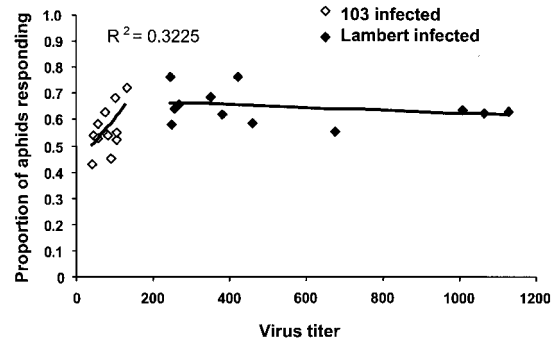


**Fig. 5.** Proportion of aphids responding in a choice test relative to virus titer levels for each of 12 plants of Lambert or Lambert-derived transgenic genotype 103.1J.

tory or tactile cues. We conclude that the cues are induced by BYDV infection because aphids congregated preferentially over BYDV-infected Lambert plants compared with noninfected and sham-inoculated plants of this variety. *R. padi* showed no preference for headspace above wheat plants of the transgenic 103.1J infected with BYDV compared with headspace above noninfected or sham-inoculated wheat plants of this genotype.

This is the first report of volatile cues associated with BYDV infection of wheat plants influencing the behavior of the vector *R. padi.* Our findings also show for the first time that transgenic virus resistance in wheat plants can indirectly influence the production of volatiles, apparently making the plants less attractive or arrestant to aphids. Working with another aphid-luteovirus system, Eigenbrode et al. (2002) found volatiles cues that arrest and attract the vector *M. persicae* to PLRV-infected plants.

Total detectable volatile compounds and the majority of individual compounds collected from headspace of BYDV-infected Lambert plants were in higher concentrations than in headspace of BYDV-infected transgenic plants and higher than noninfected and sham-inoculated plants. This difference in volatile concentration detected in Lambert plants infected with BYDV, compared with plants of the Lambert-derived transgenic 103.1J infected with BYDV, is likely the result of the response of these plants to virus infection. Our findings suggest that responses of aphids are influenced by the relative susceptibility or resistance of wheat genotypes to the virus. Lambert exhibited higher virus titer and greater susceptibility to BYDV than 103.1J, which likely affected volatile profile and concentration. Examination of responses to individual 103.1J plants relative to virus titer per plant showed a greater proportion of aphids responded as virus titer increased, suggesting volatile production may increase as virus titer level increases up to a threshold point. Additional studies are required to confirm this hypothesis. It is not known at this point if the volatiles produced from infected plants are arrestants or attractants to *R. padi.* Specific arrestants or attractants for *R. padi* to BYDV-infected plants have not been identified. This question could be addressed in further studies using leaf models and extractable headspace volatiles, as done by Eigenbrode et al. (2002) while working on the potato-PLRV-*M. persicae* system. The behavioral activity induced by specific components and the possible importance of their ratios in the headspace of BYDV-infected plants remain to be determined. From the volatiles that we identified from infected plants, (Z)-3-hexenyl-acetate was present in substantially and significantly higher concentrations in the headspace of infected Lambert plants. This is a good candidate to be tested as an arrestant or attractant to *R. padi* in further studies.

The implications of these findings for BYDV epidemiology are uncertain. McElhany et al. (1995) used a spatially explicit computer simulation of the spread of BYDV to explore the effects of vector preference for noninfected or BYDV-infected plants on disease dynamics. Their results indicated that vector preference for BYDV-infected plants should promote virus spread through a plant population when the initial proportion of infected plants is low, but retard virus spread when the initial proportion of infected plants is high. Based on this prediction, and if aphids respond to volatiles from infected plants in the field as they do in the laboratory, volatile-mediated preference for infected plants will have complex but detectable effects on the epidemiology of BYDV. Our test was conducted with plants at a single developmental stage and a single stage of disease development. Inferences to the field await further tests with a wider range of plant ages and stages in disease progression. Responses of aphids to dynamic volatile cues could also be dynamic in nature. We used apterous aphids in this study to simplify our bioassay, but responses of alates to volatiles of BYDV-infected plants need to be determined.

It is possible that *R. padi* has adapted to respond to volatile cues emitted by BYDV-infected plants because these cues signal a superior host. Feeding on BYDV-infected plants enhanced the life history of aphid vectors (Araya and Foster 1987, Fereres et al. 1989, Quiroz et al. 1991, Jiménez-Martínez et al. 2004).

Aphid physiological responses (Jiménez-Martínez et al. 2004) and behavioral responses (this study) to infected 103.1J would have implications for disease epidemiology if this type of transgenic resistance to BYDV were to be deployed. Intrinsic rate of increase of *R. padi* was lower on BYDV-infected 103.1J plants than on infected Lambert (Jiménez-Martínez et al. 2004). Additionally, *R. padi* was found to be a less efficient vector after acquiring BYDV from 103.1J compared with Lambert (Jiménez-Martínez 2003). Results from our present studies suggest that a smaller number of aphids will be attracted to moderately resistant 103.1J plants infected with BYDV than to susceptible BYDV-infected plants. The combination of reduced vector population growth on infected 103.1J plants (Jiménez-Martínez et al. 2004), together with reduced attraction to virus-infected 103.1J plants (this study) and reduced transmission efficiency after acquisition from virus-infected 103.1J plants (Jiménez-Martínez 2003), could reduce secondary transmission and virus incidence in the field (Irwin and Tresh 1990).

Knowledge of the volatile cues that influence the responses of aphids to BYDV-infected wheat plants would be useful in understanding vector behavior and movement. This could possibly lead to new methods for the control of *R. padi.* Once identified, *R. padi* arrestants or attractants produced by BYDV-infected plants could be used to reduce the spread of BYDV by artificially reducing within-field movements by the aphid vector or by selecting wheat genotypes that produce stronger or more persistent volatiles when infected with BYDV, which can be used as "trap plants." Our results show that volatile emissions from BYDV-infected wheat plants can affect one of its main vectors and suggest that further examination of other BYDV vectors or other insect vector-plant-virus relationships is warranted.

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